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The Prevalence of Aliphatic Delta-Lactones or Their Precursors in Animal Fats

P. S. Dimick, S. Patton, J. E. Kinsella and N. J. Walker,[1] Department of Dairy Science, The Pennsylvania State University, University Park, Pennsylvania

ABSTRACT

Data are presented to show the occurrence of saturated aliphatic delta lactones, namely the δ-C_{10}, δ-C_{12}, δ-C_{14}, and δ-C_{16}, in numerous ruminant and monogastric animal fats. These trace components were isolated by silicic acid adsorption chromatography followed by identification employing gas chromatography. The general prevalence of the delta-lactones or their precursors in animal depot fat, mammary tissue, blood serum lipids and milk fat is suggestive that they occur commonly in animal fats and are related to lipid metabolism.

INTRODUCTION

NUMEROUS INVESTIGATIONS from our own and other laboratories have provided evidence of γ- and δ-aliphatic lactones in bovine milk fat (1,6,8,14,17). The occurrence of these minor components has been particularly associated with the flavor of heated and stored forms of fat-containing dairy products. Evidence indicates that the precursors are monohydroxyalkanoic acids in esterified glyceride form (1,6,7,11) and that the lactones result by a spontaneous nonoxidative mechanism (1,15) involving hydrolysis and lactonization of these hydroxy acids. For better definition of the metabolic origin and significance of these lactones (hydroxy acid), it was of interest to determine whether their occurrence is limited to ruminants. The following experiments indicate general prevalence in animal fats.

EXPERIMENTAL PROCEDURE

A description of the samples analyzed and the methods of lipid extraction is presented in Table I. In order to isolate the lactone-rich fraction, silicic acid adsorption chromatography was employed. A similar technique has been reported (6). Twenty grams of Mallinckrodt silicic acid (5) was slurried onto an 18-mm I.D. glass column in ethyl ether. The column packing was washed thoroughly with 500 ml of petroleum ether (bp 35–42C). Two to six grams of lipid, dissolved in petroleum ether, was applied and washed into the packing. Elution was carried out by adding 250 ml of 100% petroleum ether, 250 ml of 10% ethyl ether in petroleum ether and lastly 300 ml of 100% ethyl ether. The first fraction eluted contained hydrocarbons and traces of sterol esters. The second fraction contained essentially all the triglycerides and some nonesterified fatty acids. The 100% ethyl ether fraction was composed of traces of triglycerides, diglycerides, monoglycerides, sterols, nonesterified fatty acids, free lactones and "lactone precursor (7)." This whole fraction was evaporated to dryness on a steam bath under N_2 and saponified with 2.5 ml 7.8% KOH in ethanol by refluxing 20 min. The solution was diluted with 2.5 ml H_2O, rewarmed to a boil, cooled and extracted

[1] Fellowship student on leave from Dairy Research Institute, New Zealand.

TABLE I

Description of Samples and References to Methods of Lipid Extraction Used in This Survey of δ-Lactones and Their Precursors

Samples		Source	Reference
Cow	Milk fat	Mixed herd	(11, 16)
	Mammary tissue	Individual cow[a]	(16)
	Depot fat	Individual steer[a]	(3)
	Whole blood	Individual cow[a]	(13)
	α-lipoprotein	Holstein cow	(12)
	β-lipoprotein	Toggenburg goat	(16)
Goat	Milk fat	Hampshire ewe	(16)
Sheep	Milk fat	Individual sheep[a]	(3)
	Depot fat	Hampshire sow	(16)
Swine	Milk fat	Individual swine[a]	(3)
	Depot fat	Mixed[b]	
Human	Milk fat	Individual	(3)

[a] History of source not precisely known.
[b] Supplied by J. B. Brown, Laboratory of Physiological Chemistry, The Ohio State University, Columbus, Ohio.

TABLE II

Comparison of Retention Times Between δ-Lactones of Human Milk Fat and Those Reference Lactones on Two GC Columns

Reference lactone	Retention time relative to δ-C_{12} (actual time, min)[a]			
	Polyester column[b]		Apiezon column[c]	
	Unknown	Reference	Unknown	Reference
δ-C_{10}	0.52(4.4)	0.53(4.5)	0.45(6.5)	0.44(6.3)
δ-C_{12}	1.00(8.5)	1.00(8.5)	1.00(14.4)	1.00(14.4)
δ-C_{14}	1.98(16.8)	1.99(16.9)	2.27(32.7)	2.26(32.6)
δ-C_{16}	3.86(32.8)	3.88(33.0)[d]	5.08(73.1)	5.07(73.0)[d]

[a] Actual retention times are measured from solvent front, samples and references run consecutively.
[b] 10% diethyleneglycol adipate plus 2% H_3PO_3 at 172C, argon pressure of 16 psig, Barber-Colman Model 10 GC equipped with radium 226 detector source. Detector cell voltage 750.
[c] 20% Apiezon-L column at 207C, argon pressure of 30 psig, Barber-Colman Model 10 GC equipped with a radium 226 detector source. Detector cell voltage 1000.
[d] Reference unavailable; time evaluated from semilog plot of retention data for homologous series of δ-lactones.

3 times with hexane to remove nonsaponifiable material. The resulting soap solution was decomposed with 6 N HCl and extracted 3 times with hexane. Upon evaporation the residue had a strong fatty acid and coconut-like (lactone) odor. This residue, dissolved in petroleum ether, was then applied to a second 20-g silicic acid column and fractions eluted as before. The bulk of the fatty acids were eluted with the 10% ethyl ether in petroleum ether fraction and the lactones were eluted from the column with pure ethyl ether. This lactone-rich fraction was then evaporated to 200 μl and 10 μl amounts were used for the gas chromatographic (GC) analysis (2). When sufficient lipid material (100 g) was available, the lactones were also isolated by steam deodorization as previously reported (17).

Identification of the lactones was accomplished by comparing GC retention times for authentic compounds (kindly supplied by J. Boldingh, Unilever Ltd., Vlaardingen, The Netherlands) with those for the unknowns on both polar (polyester) and nonpolar (Apiezon) coated column packings (for representative data, see Table II). Coincidence of the lactone odor with the emerging peak was also used as evidence of identity. However, δ-C_{16}, being unavailable to us and apparently nonodorous, was implicated by a plot of retention data for the homologous series of δ-lactones and by previous identification of it from bovine milk fat (6).

RESULTS AND DISCUSSION

Analyses of the various fats proved positive for the occurrence of δ-aliphatic lactones (Table III, Figures 1 and 2). No attempt was made to quantitate the lactones in this survey; however, a quantitative visual comparison of the lactone peaks is justified and can be seen in Figure 1 for milk fats from the ruminants

TABLE III

Methods Employed in Demonstrating the Major δ-Lactones or Their Precursors in Lipids of Various Species

Sample	Lactones			
	δ-C_{10}	δ-C_{12}	δ-C_{14}	δ-C_{16}
Cow				
Milk fat	1[a], 2[b]	1, 2	1, 2	1, 2
Mammary tissue	1	1	1	1
Depot fat	1, 2	1, 2	1, 2	1, 2
δ-lipoprotein	1	1	1	
β-lipoprotein	1	1	1	1
Goat				
Milk fat	1, 2	1, 2	1, 2	1, 2
Sheep				
Milk fat	1	1	1	1
Depot fat	1	1	1	1
Swine				
Milk fat	1	1	1	1
Depot fat	1	1	1	1
Human				
Milk fat	1, 2	1, 2	1, 2	1, 2

[a] Refers to silicic acid adsorption chromatography. For details of column, see text.
[b] Refers to steam deodorization of 100 g of fat.

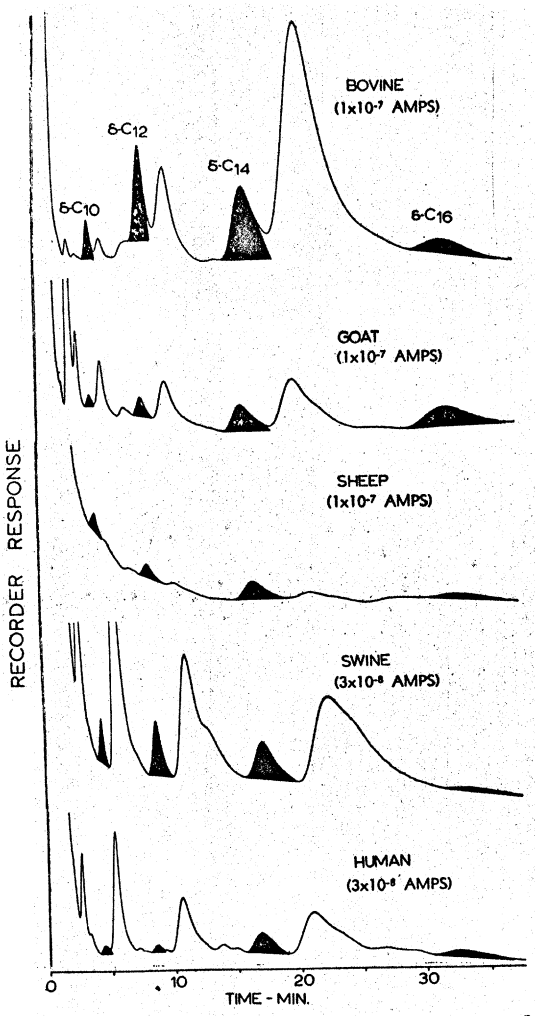

FIG. 1. Gas chromatograms of lactones isolated from the milk fats of cow, goat, sheep, swine, and human on a 6-ft by 6-mm column packed with 10% diethyleneglycol adipate treated with 2% phosphoric acid on 60-80 mesh Gas-Chrom P (Applied Science Laboratories, State College, Pennsylvania).

(cow, goat, sheep); and the monogastrics (swine, human). The initial amount of lipid material in each analysis was 6.0 ± 0.4 g, and conditions for isolation, concentration and GC were held constant. Published data (1,2,6) indicating that the δ-C_{10}, δ-C_{12}, δ-C_{14}, and δ-C_{16} lactones occur in the range of 10 to 60 ppm for cow milk fat may also provide a useful frame of reference.

The occurrence of lactones or their precursors in the milk fat of monogastric animals tends to eliminate the rumen as an exclusive source of the lactone precursors. Admittedly the human diet consists of large quantities of animal fats which could conceivably account for the presence of these compounds in mother's milk. However, with the identification of lactones in milk fat and depot fat of the swine in which the diet was completely devoid of animal fat, it is reasonable to conclude that these compounds are not unique to ruminants.

The δ-C_{10} and δ-C_{12} lactones have been detected in bovine tallow but at considerably lower levels than in bovine milk fat (1). Similarly in this investigation, traces of lactones were found in steer, sheep and swine depot fat. Interestingly, even though the depot fats of the animals analyzed are composed mainly of long chain fatty acids, namely 16 and 18 carbon acids (13); identifiable amounts of δ-C_{10}, δ-C_{12}, and δ-C_{14} lactones were evident. A similar inconsistency was evident in the swine milk fat. It is therefore assumed that the corresponding hydroxyalkanoic acid precursors, and their keto glyceride analogs (18), may be involved in a unique synthesis or degradation of fatty acids by four-carbon units. The use of intact four-carbon units (β-hydroxybutyrate) has been demonstrated in milk fat synthesis (9,10).

It was not surprising to find lactones in whole blood of the cow since they occur in depot fat, mammary tissue and milk fat. Analyses of equal amounts of lipid (2.5 g) from α- and β-lipoproteins of blood serum indicated a greater proportion of the lactone potential was present in the β-fraction. This is of particular interest since these β-lipoproteins are major contributors of lipids to milk fat (4).

From these data it is evident that the lactone precursors occur commonly in animal fats and are related to general lipid metabolism. The mechanism involved in formation of the hydroxyalkanoic acid precursors is presently under investigation.

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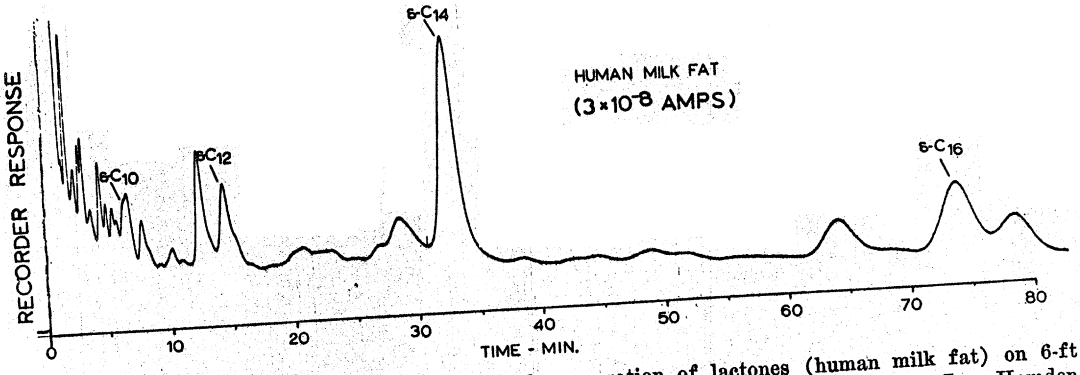

FIG. 2. Typical gas chromatogram showing the separation of lactones (human milk fat) on 6-ft by 4-mm column packed with 20% Apiezon-L on 120–140 mesh Anakrom AB (Analabs Inc., Hamden 18, Connecticut).

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